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In a similar model, alternating electric fields are shown to perturb the distribution of charged intermembraneous components in a manner that is not reversed in a single cycle and while the induced dipole moment of the cell after several complete cycles is small, the induced quadrupole moment is much more significant.

The effect of various physical parameters within these models will be shown.

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A Model for Some of the Effects of an Externally Applied Electric Field on Charged Membrane Constituents

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Abstract

It has been demonstrated that externally applied electric fields perturb the distribution of some of the molecular components of biological membranes. Various electrostatic, hydrodynamic, and structural forces that result from the application of the external field influence both the dynamics and equilibrium distribution of intermembranous molecules. From a model we have shown that the electrostatic force arising from the polarization of the counterion distribution will have a significant effect on the migration of charged membrane components. For small fields this effect on migration is independent of cell size and inversely related to temperature. In a similar model, alternating electric fields are shown to perturb the distribution of charged intermembraneous components in a manner that is not reversed in a single cycle, and while the induced dipole moment of the cell after several complete cycles is small, the induced quadrupole moment is much more significant. The effect of various physical parameters within these models will be shown.

Introduction

The use of electromagnetic energy is one of the principal features of modern technology. Electric and magnetic fields are a by-product of this technology. As the number and type of man-made sources of electric fields proliferate, more complex extraneous electric field patterns are introduced into the environment. Further, exposure to these physical agents is increasing as a result of new diagnostic and therapeutic applications [1,2]. The potential for greater and more complex exposure to electric fields makes it important to understand the mechanisms of interaction between electric fields and biological systems and the possible biological effects of these agents.

Electric fields influence biological systems through their charge structure by affecting the orientation of nonsymmetric charge distributions or altering charge distribution. Biologically generated electrical phenomena are an important component of the interaction between biological elements at various levels of organization [3–6]. While formally the interaction appears similar for the various levels (molecular to macroscopic) of organization, the size and nature of the charge carrying elements make the considerations with respect to extraneous electric fields dependent on the level of organization. On the molecular level where fields play an important role in identification processes and stability, the relevant fields have a much greater magnitude and are spatial more complex

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than extraneous fields. Except in unusual circumstances [7] it is difficult to postulate a mechanism for the interference of (environmentally relevant) fields with molecular level biological processes, where the energy gained by orienting molecular charge distributions or redistributing charge on a molecular scale is small with respect to random fluctuations. On the other hand, it has been demonstrated that fields much smaller than those needed to perturb biomolecular processes affect the function of biological systems at macroscopic levels of organization [8,9].

In the computational studies presented here, the cellular level has been selected as the organizational level to be modeled because (1) some of the underlying mechanisms for the interaction of electric fields with more macroscopic biological systems can be demonstrated at the cellular level; (2) a simple formal description of the processes is available that can be scaled to consider other more complex levels of organization; (3) (and perhaps more significantly) cellular level experiments have demonstrated that externally generated electric fields can change the distribution of charge carrying elements in a manner that may affect biological function [6,10].

It has been shown experimentally [11,12] that electric fields from external sources perturb the spatial distribution of some of the functional components in biological membranes. This effect has been demonstrated with fields smaller than 1 V/cm and field duration of a few seconds. However, most of the results are for fields of 1–10 V/cm that are unidirectional for many minutes. Experimental conditions are such that the change in the spatial distribution of these membrane components is a result of their movement within the cell membrane (polarization, altering the charge distribution) and not a change in the orientation of the cell (charge distribution orientation) or destruction and reformation of the components. These studies [6] have been performed primarily with two surface receptors, the Con A receptor and the acetylcholine receptor. While the charge on these receptors has not been measured under the experimental conditions, it has been suggested that they must be polyelectrolytes with a charge equivalent to 1–10 electronic charges and the local ionic distribution around the moving receptor should play an important part in the final receptor distribution [13]. We have been performing model computational studies to determine the importance of the counterion distribution near the receptor on its movement in an electrical field. More relevant to the environmental circumstances, we have also been modeling the effect of low-frequency alternating fields on the movement of charged particles in the cell surface; when due to the small diffusion coefficient for this motion, they do not migrate a significant distance in a single half-cycle.

Model

The cell is assumed to be a hemisphere fixed on a surface. The quantitative results depend on the shape of the cell, but the most significant qualitative conclusions do not. The immobile cell is consistent with the experimental literature. Initially the mobile cell surface receptors are equally distributed over

the surface. They are free to move parallel to the cell surface, but any force perpendicular to the surface will be counter balanced by the various structural forces of the membrane. The number of mobile membrane bound charged molecules is small with respect to the total membrane bound charge. The cell is in an ionic milieu, and as there is no information about the specific ion site or the charge distribution of the receptor, the distribution of mobile ions near the receptor is assumed to depend only on the distance from the cell surface when there is no externally applied field. Any ions bound to the receptor (not mobile) are considered part of the receptor. The layer of ions near the cell surface will be polarized by an external field and reach its final distribution in a time that is short with respect to any movement of the membrane bound receptors [14,15].

The field is applied parallel to the surface on which the cell is fixed. It is much smaller than the radial field generated by the cell surface charge, but has a component perpendicular to that field (see Fig. 1).

The Effect of Counterions on the Migration of Charged Receptors in a Unipolar Field

The additional potential outside the cell due to the externally applied field is [14]

$$V(R,\theta) = -E_0 R \cos\theta + f(K)a^3 \cos\theta / R^2 , \tag{1}$$

where R and θ are the radial and polar spherical coordinates in the cellular coordinate system. a is the radius of the cell and $f(K)$ is a function of the conductivities of the counterion layer and the cell surface [$f(K) \sim -0.5$ for all cases of interest].

The charge distribution outside the cell due to the counterions considered is

$$\rho(R,\theta) = \sum_i \rho_{0i}(R) e^{-q_i V(R,\theta)/kT}, \tag{2}$$

where the sum i is over all types of counterions; $\rho_{0i}(R)$ is the counterion charge distribution when no external field is applied. It has been taken as a Boltzmann distribution using the Gouy–Chapman potential as the unperturbed radial potential* (lacking more specific information), q_i is the charge of the ith ion, k is Boltzmann's constant, and T is absolute temperature.

A screened interaction potential using the standard parameters for bulk water has been assumed, in order to insure an underestimation of the electrostatic interaction between the counterions and the charged, mobile membrane-bound molecules. To further insure an underestimate and, therefore, to provide a lower bound for the relative importance of this interaction in comparison with other forces, the initial distribution of counterions is taken as the difference between

*The problems with using Gouy–Chapman theory have been discussed previously, but it has been observed that for lipid vesicles it provides a good description of some relevant experimental results [16].

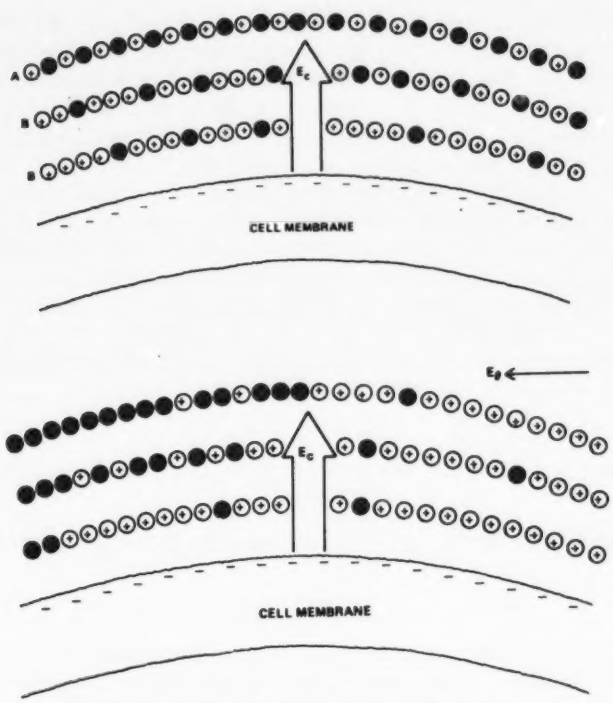

Figure 1. An illustration of the polarization of the counterion distribution near the cell surface by an external electric field. (A) Before the field is applied, the counterion distribution depends only on the distance from the cell surface. There is a large naturally occurring electric field that always is perpendicular to the cell surface. (B) During the exposure to the external electric field, the counterion distribution is polarized. The applied field is much smaller than the natural field but has a component perpendicular to it.

the positive and negative ion distribution (derived from Gouy–Chapman theory) and the center of a counterion is assumed to come no closer than 3 Å to the center of a charged surface particle. These last assumptions are more conservative than ref. 13 and more likely to underestimate the interaction (for more details, see [13]).

For $qaE_0 < kT$, where q is the charge of the counterion (note the charge on the membrane bound receptor multiplied by aE_0 may not be smaller than kT), the electric field (E_θ) at the cell surface, due to the polarization of the ith counterion distribution is

$$E_\theta = \tfrac{3}{2} \int \rho_0(R) \frac{e^{-\kappa r}}{\epsilon r} d\tau \frac{qE_0 \sin\theta'}{kT} + \cdots, \tag{3}$$

where the integration is over the half-space 3 Å or more from the hemisphere

and ρ_0 (R) is a function of the cell surface potential. κ and ϵ are the screening parameter and dielectric constant. This field is parallel to the cell surface (see [13] for more details).

The Effects of an Alternating Electric Field on the Distribution of Charged Mobile Intramembranous Particles

The alternating field is assumed to vary in a stepwise manner, and for computational purposes the steps may be as small as desired. For each step the particle distribution decays from its initial state toward a final state. The final state is the state the distribution would be in if it had enough time to come to equilibrium at that field strength. As there is not enough time, the state at the end of a step contains a small amount of the final state added to the initial state. The amount of the final state added depends on the relaxation time for this movement and the duration of the step. The calculation of the relaxation time assumes there is no barrier between the initial and final states (for details, see [17]).

Results

E_θ in eq. (3) has been estimated in two different ways. In each case a large number of hemispherical shells about the point of interest on the cell surface are summed and each shell is assumed to have a constant charge density (i.e., the charge density is determined by the proximity to a surface charge and the changes due to the external field will be small in comparison with the effects due to the surface charge). Case A uses a minimum density for the shell with regard to our previous assumptions [$\rho(R) = \rho(R_{max})$]. Case B uses a mean value [$\rho(R) = \rho(\bar{R})$].

Figure 2 gives E_θ in units of $E_0 \sin\theta$ as a function of cell surface potential in 0.15 molar salt solution for surface potentials less than $2kT/q$. It can be seen that even for these minimizing assumptions E_θ is a significant fraction of E_0 at all surface potentials of interest. E_θ decreases with increasing temperature. The effect of bivalent ions increases as the surface potential increases.

The results for the effects of alternating electric fields on the distribution of charged mobile intramembranous particles can be seen in Figures 3 and 4. Figure 3 is for a set of input parameters that are chosen to be reasonable but show maximum movement. It is striking to note that after a single cycle the distribution of particles has increased at both ends of the cell and decreased in the middle. It has increased at surfaces near perpendicular to the field and decreased for surfaces parallel to the field. The distribution does not return to its initial state at the end of a complete cycle.

In Figure 4 the input parameters were chosen so the movement during a single cycle would be smaller than in the previous example. The change in the density of charged intramembranous particles in a 30° cap about the ends of the cell (i.e., the surfaces nearly perpendicular to the field) are shown. The particle density continues to increase for the 100 cycles investigated.

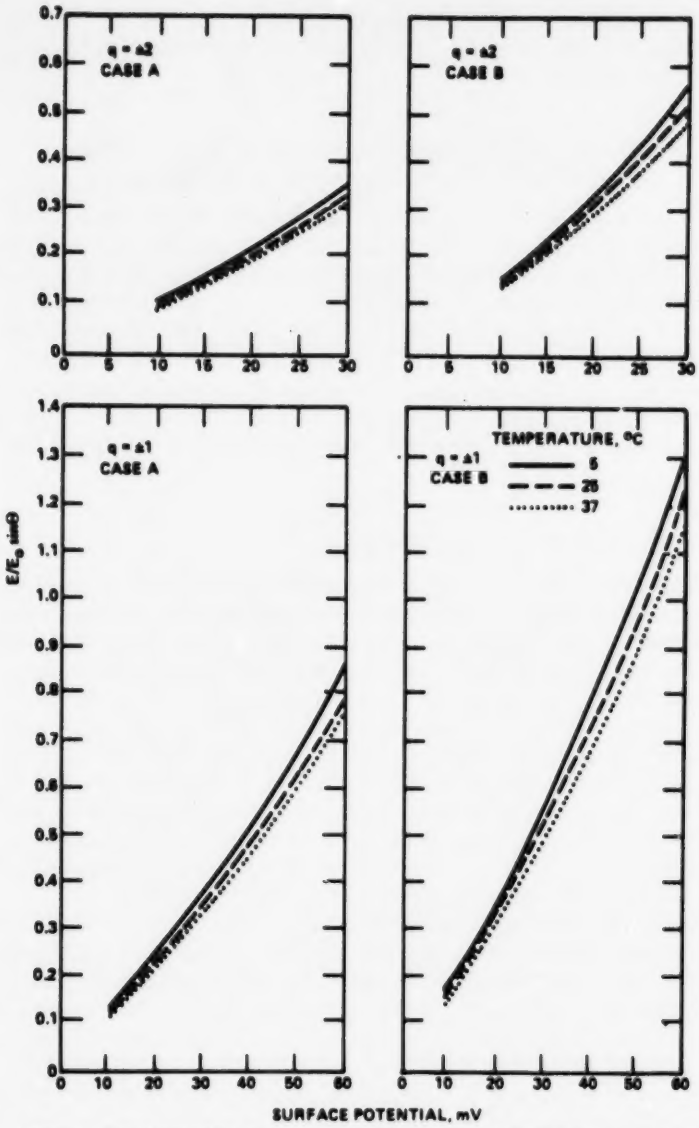

Figure 2. The electric field due to counterion distribution polarization for monovalent and bivalent counterions with an initial charge distribution that underestimates the effect. Curves are shown for two alternatives in the evaluation of eq. (3). Case A is an underestimation.

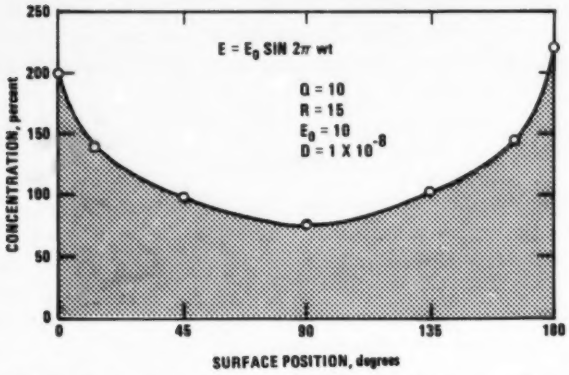

Figure 3. The distribution of charged intramembranous particles after a single cycle of an externally applied alternating electric field. It is shown as a function of position on the cell surface. The angle shown is also the angle a normal to the surface would make with the field for the positive half-cycle. It is quantified as a percentage of the distribution before the field is applied. Before the field is applied the distribution is independent of the position on the cell surface. Q is in electronic charges per surface molecule, R is in μm, E_0 is in V/cm, and D is in cm^2/s.

This movement of charge toward both poles of the cell will have no effect on the dipole moment of the cell calculated about its center. It will still be very small. This movement will have a large effect on the cellular quadrupole moment, suggesting the cell will interact more strongly with inhomogeneous electric fields when in an alternating field.

The biological implications of the movement of charged mobile intramembranous particles toward both poles of a hemispherical cell in an alternating electric field will be discussed in more detail elsewhere [17]. This phenomenon

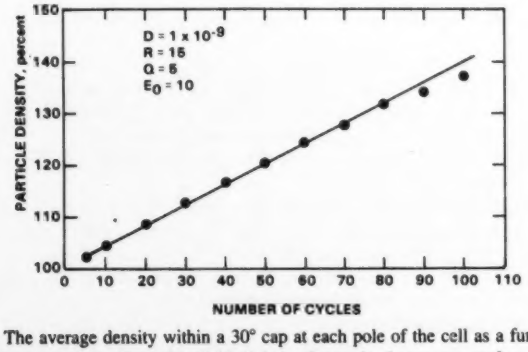

Figure 4. The average density within a 30° cap at each pole of the cell as a function of the number of cycles of the alternating field. Q is in electronic charges per surface molecule, R is in μm, E_0 is in V/cm, and D is in cm^2/s.

can be reasonably generalized to cells of different shapes (as long as they remain fixed in space) and charged particle densities will increase on surface perpendicular to the alternating electric field and decrease on surfaces parallel to the field.

Conclusions

(1) The electrostatic force that results from the polarization of the counterion distribution near the cell surface by an externally applied unipolar electric field will have a significant effect on the migration of charged membrane components.

(2) The distribution of charged mobile membrane constituents will be perturbed by an alternating electric field. It will not return to its initial value after a complete cycle and may increase over many cycles.

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